1. *What is the Clinical Proteomic Technologies Initiative for Cancer?*

   The Clinical Proteomic Technologies Initiative for Cancer (CPTI) is a five-year initiative designed to develop and assess current proteomic technology capabilities, develop well characterized resources and reagents for the scientific community, and support innovative technological and computational approaches for proteomic analysis. The CPTI is composed of three main components:
   - Clinical Proteomic Reagents Resource
   - Advanced Proteomic Platforms and Computational Sciences
   - Clinical Proteomic Technology Assessment for Cancer.

2. *What role can proteomics play in cancer research?*

   It is believed that cancer-related peptides and proteins will be secreted into and circulate through the bloodstream as normal cells transform into cancer cells. These peptides and proteins will serve as sentinels of the disease. Through identification and characterization of these proteins, scientists and clinicians will be able to diagnose and treat cancer at its earliest stages. However, it is estimated that the dynamic range of cancer-related proteins may be as low as 10^{10} that of the most concentrated proteins such as albumin and hemoglobin.

3. *Why is the focus of this NCI proteomics program on technology and resource development and assessment, and not on biomarker discovery?*

   Effective biomarker discovery efforts for clinical cancer proteomics will require an increased ability to identify cancer-related proteins that exist in a low concentration within the blood. Current technologies lack the capability to reproducibly identify, quantify, and validate such proteins. This initiative is designed to develop the necessary reagents for proteomic research and enhance the current capabilities to conduct this research in a reproducible manner so that candidate biomarker proteins can be identified and verified across labs.

4. *What does the Clinical Proteomic Technology Assessment for Cancer (CPTAC) intend to accomplish?*

   The CPTACs will establish a multidisciplinary network that will conduct rigorous proteomic technology assessment; develop standard protocols and clinical reference sets; and evaluate methods to ensure data reproducibility. As such, the CPTACs will develop well-characterized standards and protocols for research platforms. The CPTACs will conduct a rigorous assessment of these platforms to determine sources of variability for all the steps from sample acquisition to data analysis and storage. Areas of assessment will include sample acquisition, preparation, fractionation, labeling, and peptide/protein detection; and data acquisition, analysis, and interpretation. The CPTACs will focus on

experimental design, methods, standards development, data analysis, and inter-laboratory comparisons. By the end of the five-year initiative, it is the goal of the CPTACs to have documented sources of variability in experimental protocols, highly annotated reference standards for instrument calibration and measurement, along with the protocols, experimental resources, and clinical reference sets necessary for conducting reproducible experiments.

5. *Is the Clinical Proteomic Technologies Initiative associated with the NIH Roadmap?*

   The NIH Roadmap outlines under its **New Pathways to Discovery** a key component, *Standards for Proteomics and Metabolomics/Assessment of Critical Reagents for Proteomics*. While the CPTAC is not instituted within the Roadmap programs, the CPTAC will complement the Roadmap goals in proteomics. The CPTAC is focused on assessing proteomic technologies for application in clinical cancer research. This will include the development and characterization of standards, as detailed in the Roadmap, but the CPTAC will encompass the standardization of protocols and data analysis focused on identifying and validating cancer-specific biomarkers. This will include optimizing procedures for capturing and identifying proteins within the dynamic range thought to be associated within tumor tissue leakage (below pg/mL concentrations) from plasma. In addition, the CPTAC will incorporate both mass spectrometry and affinity capture technologies (such as protein microarrays) to develop appropriate procedures for both discovery and validation research.

6. *How much will the NCI invest in this initiative?*

   The entire Clinical Proteomic Technologies Initiative for Cancer is a five-year, $104 million initiative. Of that, the CPTAC program will be $35.5 million over five years, with approximately $8.5 million invested in the first year.

7. *Who did the NCI consult in developing this initiative?*

   The Clinical Proteomic Technologies Initiative has been under development since 2002 when a workshop was co-hosted by the NCI, NHGRI, and NIGMS. Subsequent workshops were held to help shape the initiative including two roundtable *Clinical Proteomics and Biomarker Discovery in Cancer Research* meetings led by Nobel Laureate and president of the Fred Hutchinson Cancer Research Center, Lee Hartwell, and attended by leading world experts in proteomics and cancer biology. Through these and other meetings it became apparent that a critical shortfall in cancer proteomics was the inability to reproduce and validate experimental data. In consultation with the NCI's Board of Scientific Advisors, an advisory group composed of extramural scientists and clinicians, the CPTAC program was developed to address the need for proteomic technology calibration, standardization, and validation.

8. *What other funding opportunities exist within the Clinical Proteomic Technologies Initiative for Cancer?*

   The CPTAC program is intended to conduct a rigorous assessment of proteomic technologies. To assist in this effort, the Clinical Proteomic Technologies Initiative for Cancer will support a *Clinical Proteomic Reagents Resource* and an *Advanced Proteomic Platforms and Computational Sciences* program. The Clinical Proteomic Reagents Resource will support the development and characterization of peptides, proteins, antibodies, and standards through various contracting mechanisms. These resources will be provided for use by the broader scientific community for application in proteomic research. The Advanced Proteomic Platforms and Computational Sciences program will use R01 and R21/R33 funding mechanisms to support innovative proteomic technology and data analysis development. Both of these programs will be incorporated into the CPTAC program throughout the five-year effort. In addition, the NCI has other funding opportunities in proteomic research through its Innovative Molecular Analysis Technologies (IMAT) programs that may also assist in advancing proteomic technologies.

9. *What are the critical components and minimal requirements necessary for applying for the Clinical Proteomic Technology Assessment for Cancer RFA?*

   The CPTAC program is intended to be conducted as a series of carefully coordinated experimental designs to evaluate all components of proteomic platforms in cancer research from sample acquisition to data analysis and interpretation. The CPTAC participants will need five core components of expertise: cancer molecular biology, clinical research, statistical (both experimental design and data analysis), computational sciences, and proteomic technology specialties. These components do not have to be represented to an equal degree but it is expected that they will each have roles throughout the program's lifespan. It is important that each component is incorporated beginning with the experimental design stage to develop plans for the appropriate procedures to achieve intended results. Each CPTAC team is required to have a minimum of two mass spectrometry platforms, of which one platform must have at least two machines. In addition, each team must include an alternative proteomic technology, such as protein microarray, to complement and validate mass spectrometry data. Applicants are encouraged to incorporate private sector institutions as components or participants of their teams if it will strengthen the program.

10. *Can foreign institutions apply?*

    Foreign institutions cannot apply as the Principal Investigator (PI), but may participate in a team with a domestic PI as a partner or sub-contractor.

11. *Can one institution play a role in multiple teams within the CPTAC program?*

    Yes. However, each applicant may only apply as the PI on one application. Other institutions may serve as partners or sub-contractors on multiple teams. For example, a group with expertise in mass spectrometry computations and data analysis may serve as the data analysis component on multiple teams.

12. *What technologies will be required for application to the CPTACs? What technologies will not be considered for evaluation through the CPTACs?*

    The purpose of the CPTAC program is to assess proteomic technology for peptide/protein discovery, validation, and clinical application approaches. Since it is not intended as a biomarker discovery effort, the technologies must be able to analyze samples and identify proteins in a high-throughput and reproducible manner on human clinical samples. The CPTAC teams will be required to have the capacity and expertise to analyze samples on at least two different mass spectrometry platforms (matrix assisted laser desorption ionization (MALDI), electrospray ionization (ESI), time-of-flight (TOF), Fourier transform ion cyclotron resonance (FTICR), and tandem MS/MS). In addition, a complementary technology such as protein microarrays will also be required. While a variety of fractionation procedures will be adequate for consideration, the procedures must be high throughput and reproducible. Labeling technologies must be able to be applied to human clinical samples. SELDI MS will not be considered as an appropriate proteomic technology due to its limitations with probing into the dynamic range needed for cancer specific proteins and limitations in peptide/protein identification. Likewise, 2-Dimensional gel electrophoresis will not be considered due to its slow throughput, limitations in isolating proteins at low concentrations, and poor reproducibility.

13. *What is the U24 funding mechanism and how does it differ from traditional grants?*

    The U24 funding mechanism is a cooperative agreement mechanism whereby the NCI will sit as one of the partners in the CPTAC. Each PI, or appropriately designated appointee, will make up the other partners that will be part of the Program Coordinating Committee (PCC), the management body that oversees and guides the CPTAC program. Since this RFA will be instituted as a cooperative agreement, each applicant will be required to develop a set of milestones, which they intend to achieve throughout the program. The PCC will evaluate each of the teams and their progress towards achieving the milestones and work with the teams to develop best practices towards achieving the program goals.

14. *What is the sharing plan required for the CPTAC program participants? What role does intellectual property (IP) have in the initiative?*

    Each team will be required to share materials, protocols, data, samples, algorithms, and resources with the other teams in the CPTAC program. All the data, standards, samples, reference sets, and other resources developed through the CPTAC program will also have to be made publicly available throughout the project. Applicants will be required to develop a sharing plan as part of the application, which will detail how the data and materials are intended to be shared and a timeline with which the data will be made available. The NIH policy for data sharing can be found at http://grants.nih.gov/grants/policy/data_sharing. All algorithms will be required to be made open source code and data will be distributed in a preprocessed format. The NCI recognizes that IP rights may be associated with specific technology components of the program. However, it is expected that the inventors will promote wide accessibility to these research tools to other teams within the CPTAC as well as the broader scientific community.

15. *What is the role of caBIG in the initiative?*

    The cancer Bioinformatics Grid (caBIG) (http://cabig.nci.nih.gov) is a network or grid developed by the National Cancer Institute's Center for Bioinformatics that will work with investigators in cancer research to develop an infrastructure for sharing data and information. caBIG itself is not part of the Clinical Proteomic Technologies Initiative for Cancer. But it is expected that participants in the CPTAC and associated programs will work with caBIG to make experimental resources and data, and their associated ontologies and annotations, compatible with caBIG guidelines. caBIG will serve as a centralized bioinformatics platform to exchange information within the CPTAC and scientific communities. caBIG will work with each of the participants within the CPTAC program to coordinate efforts and align the bioinformatics components for compliance with caBIG.

16. *What biological specimens will be analyzed through the CPTAC program?*

    Each applicant will propose between 1-3 cancer types to be used in their proteomic platforms. Each cancer type must have at least 200 individual prospectively collected clinical samples of body fluid from individuals diagnosed with cancer and the matching tumor tissue if it has been surgically removed. The biological samples will be collected in coordination with procedures developed through the NCI's Office of Biorepositories and Biospecimen Research (http://biospecimens.cancer.gov). In addition, the NCI encourages applicants to include an appropriate mouse model of cancer so that experimental designs can be developed without using valuable clinical samples. To assist in this process, the NCI will also be providing a common mouse model of cancer for use across all participating teams.

17. *What steps will be taken to develop standards and protocols for the CPTAC? How will the CPTAC program share data, protocols, and resources, and how will the optimal strategies be determined?*

    Each CPTAC team will develop its own proteomic analysis platform for the application. The CPTAC teams will work through the Program Coordinating Committee to align strategies and protocols to assess the different platforms, determine experimental sources of variability, and optimize approaches to experimental designs. The NCI will provide protein mixtures and prepared sample biological fluids (e.g., plasma) to the teams as measurement assessment materials. Each of the teams will be required to measure the content of these mixtures and experimental results will be compared across the different sites. Various endpoints may be used to determine optimized strategies, but the primary evaluation will be focused on the number of peptides/proteins reproducibly identified. The teams will be required to share protocols and data with the other members of the program. The NCI will help coordinate meetings and workshops among the team members to compare these strategies and determine optimal approaches. Each team will also be required to store sufficient quantities of biological samples to share with the other teams in the program. Appropriate storage and shipping conditions will be determined by the Program Coordinating Committee as a component of the program.

18. *How will the data and results be disseminated? What opportunities will exist for publications or national prominence?*

    A major component of the initiative will be the development of a database to store all of the preprocessed data for availability to the scientific community. The teams will work with the NCI and caBIG to develop strategies and common data elements for storing and sharing this data. In addition, the teams will work with the NCI to develop timelines for making the data publicly available. There will be many opportunities for publication, but the focus of the manuscripts will be on protocols and standardization and not biomarker discovery. Since each team will be an evaluation center, it is expected that they will also serve as a training center for proteomics researchers to learn appropriate procedures and best practices.